FIELD EVALUATION OF DIFFUSIBLE SIGNAL FACTOR PRODUCING GRAPE FOR CONTROL OF PIERCE'S DISEASE

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ABSTRACT
A cell density-dependent gene expression system in *Xylella. fastidiosa* (*Xf*) mediated by a small signal molecule called diffusible signal factor (DSF) which we have now characterized as 2-Z-tetradecenoic acid (hereafter called C14-cis) controls the behavior of *Xf*. The accumulation of DSF attenuates the virulence of *Xf* by stimulating the expression of cell surface adhesins such as HxfA, HxfB, Xada, and fimA (that make cells sticky and hence suppress its movement in the plant) while down-regulating the production of secreted enzymes such as polygalacturonase and endogluconase which are required for digestion of pits and thus for movement through the plant. Artificially increasing DSF levels in plants in various ways increases the resistance of these plants to Pierce's disease (PD). Disease control in the greenhouse can be conferred by production of DSF in transgenic plants expressing the gene for the DSF synthase from *Xf*; such plants exhibit high levels of disease resistance when used as scions and confer at least partial control of disease when used as rootstocks. This project is designed to test the robustness of disease control by pathogen confusion under field conditions where plants will be exposed to realistic conditions in the field and especially under conditions of natural inoculation with insect vectors. We are testing two different lineages of DSF-producing plants both as own-rooted plants as well as rootstocks for susceptible grape varieties in two field sites. Plants were established in one field site on August 2, 2010. Disease severity and population size of the pathogen will be assessed in the plants as a means of determining their susceptibility to PD.

LAYPERSON SUMMARY
Xylella. fastidiosa coordinates its behavior in plants in a cell density-dependent fashion using a diffusible signal factor (DSF) which acts to suppress its virulence in plants. Artificially increasing DSF levels in grape by introducing the *rpfF* gene which encodes a DSF synthase reduces disease severity in greenhouse trials. We are testing two different lineages of DSF-producing plants both as own-rooted plants as well as rootstocks for susceptible grape varieties. Disease severity and population size of the pathogen will be assessed in the plants as a means of determining their susceptibility to Pierce's disease.

INTRODUCTION
Our work has shown that *Xylella fastidiosa* (*Xf)* uses diffusible signal factor (DSF) perception as a key trigger to change its behavior within plants. Under most conditions DSF levels in plants are low since cells are found in relatively small clusters, and hence cells do not express adhesins that would hinder their movement through the plant (but which are required for vector acquisition) but actively express extracellular enzymes and retractile pili needed for movement through the plant. Disease control can be conferred by elevating DSF levels in grape to "trick" the pathogen into transitioning into the non-mobile form that is normally found only in highly colonized vessels. While we have demonstrated the principles of disease control by so-called "pathogen confusion" in the greenhouse, more work is needed to understand how well this will translate into disease control under field conditions. That is, the methods of inoculation of plants in the greenhouse may be considered quite aggressive compared to the low levels of inoculum that might be delivered by insect vectors. Likewise, plants in the greenhouse have undetermined levels of stress that might contribute to Pierce's disease (PD) symptoms compared to that in the field. Thus we need to test the relative susceptibility of DSF-producing plants in the field both under conditions where they will be inoculated with the pathogen as well as received "natural" inoculation with infested sharpshooter vectors. We also have recently developed several new sensitive biosensors that enable us to measure *Xf* DSF both in culture and within plants. We could gain considerable insight into the process of disease control by assessing the levels of DSF produced by transgenic *rpfF*-transformed grape under field conditions.

OBJECTIVES
1. Determine the susceptibility of DSF-producing grape as own-rooted plants as well as rootstocks for susceptible grape varieties for Pierce's disease.
2. Determine population size of the pathogen in DSF-producing plants under field conditions.
3. Determine the levels of DSF in transgenic *rpfF*-expressing grape under field conditions as a means of determining their susceptibility to Pierce's disease.

RESULTS AND DISCUSSION

Disease susceptibility of transgenic DSF-producing grape in field trials.

Field tests are being performed with two different genetic constructs of the *rpfF* gene in grape and assessed in two different plant contexts. The *rpfF* has been introduced into Freedom (a rootstock variety) in a way that does not cause it to be directed to any subcellular location (non-targeted). The *rpfF* gene has also been modified to harbor a 5' sequence encoding the leader peptide introduced into grape (Thompson seedless) as a translational fusion protein with a small peptide sequence from RUBISCO that presumably causes this RpfF fusion gene product to be directed to the chloroplast where it presumably has more access to the fatty acid substrates that are required for DSF synthesis (chloroplast-targeted). These two transgenic grape varieties are thus being tested as both own-rooted plants as well as rootstocks to which susceptible grape varieties will be grafted. The following treatments are thus being examined in field trials:

Treatment 1	Non-targeted RpfF Freedom
Treatment 2	Chloroplast-targeted RpfF Thompson
Treatment 3	Non-targeted RpfF Freedom as rootstock with normal Thompson scion
Treatment 4	Chloroplast-targeted RpfF Thompson as rootstock with normal Thompson scion
Treatment 5	Normal Freedom rootstock with normal Thompson scion
Treatment 6	Normal Thompson rootstock with normal Thompson scion
Treatment 7	Normal Freedom
Treatment 8	Normal Thompson

Treatments 5-8 serve as appropriate control to allow direct assessment of the effect of DSF expression on disease in own rooted plants as well as to account for the effects of grafting per se on disease susceptibility of the scions grafted onto DSF-producing rootstocks.

One field trial was established in Solano County on August 2, 2010. Twelve plants of each treatment were established in randomized complete block design. Self-rooted plants were produced by rooting of cuttings (about 3 cm long) from mature vines of plants grown in the greenhouse at UC Berkeley. Cuttings were placed in a sand/perlite/peatmoss mixture and subjected to frequent misting for about four weeks, after which point roots of about 10 appeared. Plants were then be transferred to one gallon pots and propagated to a height of about 1 m before transplanting into the field. Grafted plants were produced in a similar manner. 20 cm stem segments from a susceptible grape variety were grafted onto 20 cm segments of an appropriate rootstock variety and the graft union wrapped with grafting tape. The distal end of the rootstock variety (harboring the grafted scion) was then be placed in rooting soil mix and rooted as described above. After emergence of roots, the grafted plant were then transplanted and grown to a size of about 1 m as above before transplanting into the field site.

The plants have all survived and are growing well (**Figure 1**). The plants are too small to inoculate in the 2010 growing season and hence will be inoculated in 2011 (no natural inoculum of *Xf* occurs in this plot area and so manual inoculation of the vines with the pathogen will be performed. Because researchers from both UC-Berkeley and UC-Davis will be contributing treatment to each plot, and since the controls for some researchers will be the same, some control plants will be shared between research groups. All plants in Solano County will be inoculated by needle puncture through drops of *Xf* of about 10^6 cells/ml as in previous studies. Due to severe damage suffered by some plants in the greenhouse at UC Berkeley due to pesticide applications, there were not sufficient plants available to initiate the trial at Riverside County: The plants needed for this trial are being regenerated and will be ready for planting before the 2011 growing season. The plants at the Riverside County location will not be artificially inoculated, but instead will be subjected to natural infection from infested sharpshooter vectors having access to *Xf* from surrounding infected grape vines. Disease symptoms will be measured bi weekly starting at eight weeks after inoculation at the Solano County site, or about eight weeks after transplanting into the field site at the Riverside County location. Leaves exhibiting scorching symptoms characteristic of PD will be counted on each occasion, and the number of infected leaves for each vine noted. ANOVA will be employed to determine differences in severity of disease (quantified as the number of infected leaves per vine) that are associated with treatment.

Figure 1. Overview of research plot in which DSF-producing plants are established (left). Closeup of transgenic Freedom vines in mid-September (right).

CONCLUSIONS

The transgenic plants have been successfully established in one of two field sites, with the second field site to be established in late 2010. The first disease assessments should be made in 2011. Since substantial disease control has been observed in these plants in the greenhouse, these tests should provide a direct assessment of the utility of such transgenic plants for disease control in the field.

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